MICROELECTRODES WITH THREE-DIMENSIONAL STRUCTURES FOR IMPROVED NEURAL INTERFACING

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Abstract-**This paper describes the development of microelectrodes with integrated three-dimensional electrode structures. The integration of three-dimensional structures aims at an improvement of the electrode/tissue interface. Due to the increase in surface area the electrode impedance is reduced, while the density of microelectrodes per area remains the same as with flat electrodes. Two different types of electrodes have been developed: Flexible, implantable microelectrodes with pyramidal, protruding structures and tip-shaped electrode arrays on glass substrates. The protrusion heights of the electrode sites can easily be adjusted depending on the actual application. For the flexible structures we used a polyimide-based process to fabricate microelectrodes with sharp or flat pyramidal tips and with electrode arrangements on front and backside of the devices. The tip-shaped electrode arrays were fabricated from a glass substrate by isotropic wet chemical etching and subsequent metallization and passivation. Data from impedance measurements and acute brain slice recordings indicate a considerable improvement regarding electrode impedance and obtainable signal strength.**

Keywords **– Three-dimensional, Electrode array, Microelectrodes, Polyimide, Glass**

I. INTRODUCTION

Microelectrodes have gained greatest interest for studying the behavior of various types of biological tissue as they make it possible to electrically stimulate tissue and record from cells with high spatial resolution. Silicon or glass-based microelectrode arrays are often used for *in-vitro* experiments, such as recording and stimulation in acute brain slices [1]. However, when looking at the respective data, it is clear that the amplitudes of evoked responses are at least one order of magnitude lower compared to signals obtained with conventional glass microelectrodes.

For *in-vivo* experiments or prosthetic implants, a flexible structure is preferred to avoid device failure and cell damage. Polyimide-based microelectrodes with a polyimide-platinum-polyimide sandwich structure have drawn attention as highly flexible bio-interfaces [2]. Polyimides combine excellent electrical and mechanical characteristics with biocompatibility [3], and are well known in microfabrication.

The electrode/tissue interface is the critical point when bioelectric signals are recorded or tissue is stimulated with current pulses. The interface can be improved by the use of adapted materials, such as activated iridium oxide films [4], or by integrating three-dimensional (3D) electrode sites. For extracellular monitoring brain slices activity, a 3D structure slightly penetrates the dead cell layers that are created during the preparation cut [5]. Tip-shaped electrodes allow for a smooth tissue penetration in order to get closer to active cells resulting in higher signal amplitudes. This also holds valid when flexible microelectrodes with small tips are implanted for the stimulation of tissue that is arranged in layers.

Here we present the integration of 3D electrode arrangements on flexible substrates and glass-based microelectrode arrays. Flexible microelectrodes with pyramidal structures can smoothly be slid into tissue layers with only small volume displacement and incorporate contacts on front and backside. Glass-based microelectrode arrays with sharp tips were used to record bioelectric activity from brain slices. For both types, the protruding tips can gently penetrate the cell layers to enable higher recording signal strengths at lower impedance values.

II. MATERIALS AND METHODS

A. Polyimide-based microelectrodes with pyramidal tips

For the fabrication of polyimide-based electrodes with integrated 3D structures, photosensitive and non-photosensitive polyimides can be used. We will outline the fabrication process for the photosensitive polyimide. For the non-photosensitive polyimide the photolithography is replaced by dry etching techniques.

An anisotropically etched silicon wafer is used as a mold to form pyramidal shapes. For that purpose, a 100 mm diameter silicon substrate [100] was etched in KOH (silicon oxide mask) to from negative pyramidal shapes on the wafer (Fig. 1a). The etch attack is self-limiting and generates negative pyramids (Fig. 1a, left) at an angle of 57.4°. If the etch is stopped before the final depth is reached, flat tipped pyramids can be obtained (Fig. 1a, right). Following the etch procedure, layers of chrome (adhesion layer), gold and aluminum were evaporated on the substrate. The aluminum is used as sacrificial layer for the later release of the polyimide electrodes, whereas the gold is used as back electrode during the release step. On the aluminum a 4 to 10 µm thick layer of photosensitive polyimide precursor (PI-2732, DuPont) was spin-coated, photostructured and cured under nitrogen for one hour (Fig. 1b). Layers of titanium (adhesion layer) and platinum (electrode material) were sputter deposited and structured by dry etching to form the metallization layer and to cover the pyramidal molds (Fig. 1c). Finally, a second layer of polyimide precursor was spun on, photostructured and again cured under nitrogen for one hour (Fig. 1d). After fabrication the obtained microelectrodes have to be dissociated from the carrier support. We have developed a release technique involving electrochemical etching of the sacrificial aluminum layer [6]. This technique enables to detach microelectrodes even if the electrode sites on one side of the substrate are in direct contact with the fabrication support. The platinum covered pyramids or flat electrode sites will not be attacked by this technique, whereas the titanium adhesion layer will be dissolved with the aluminum due to the difference in electrochemical potentials. The platinum remains attached to the top polyimide layer.

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle Microelectrodes With Three-Dimensional Structures for Improved Neural Interfacing	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Institute of Microsystems Swiss Federal Institute of Technology Lausanne EPFL, Switzerland	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
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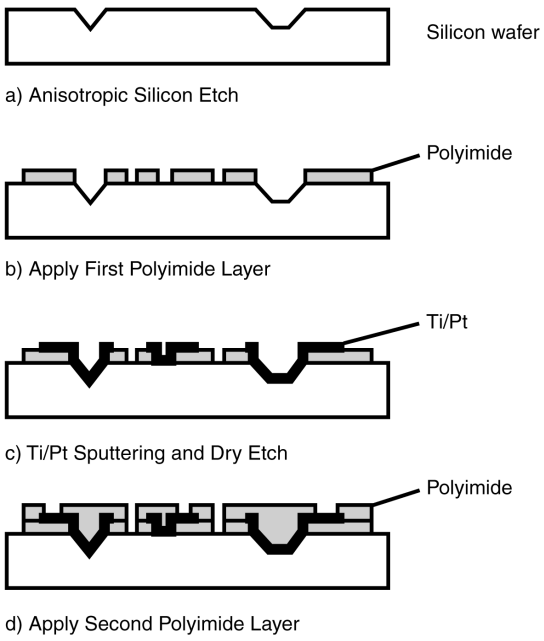

Figure 1 : Fabrication process for polyimide-based microelectrodes with pyramidal tips

B. Tip-shaped electrode arrays on glass

For the tip-shaped electrode arrays, float glass plates (thickness 700 µm, 100 mm diameter) were used as substrate material. Since most photoresists are etched or removed from substrate (bad adhesion) in HF solutions, the best way to mask glass for bulk wet chemical etching in HF solutions is the use of a metallic mask. The isotropic mask underetching occurring in HF solutions will form sharp tips at the substrate surface. Most metals deposited as adhesion layer like titanium, tantalum and chromium are etched in HF solutions. However, tests showed that chromium deposited by sputtering at high temperature (455°C) has an improved adhesion to the glass substrates and seems to resist hydrofluoric acid when covered by another metal like copper or gold, or a resistant photoresist such as SC100 resist (Olin Corporation).

First, a chromium layer (150 nm) followed by a SC100 photoresist mask were deposited and patterned onto the glass substrate. Then, the glass was etched in a 10% HF solution at 20°C until detachment of the chromium masks (about 40 to 50 minutes) in order to obtain 60 µm high glass tips on the substrate. The next step was the deposition and patterning of AZ5214 photoresist (Clariant) in order to define the negative of the electrode pattern. Deposition of a 50 nm titanium/150 nm platinum layer and photoresist removal in acetone completed the deposition of platinum electrodes onto the tips and the substrate. The last step was the deposition and patterning of a 5 µm thick SU-8 epoxy insulation layer. After chip separation by substrate dicing, the electrode arrays were assembled to a printed circuit board using a conductive glue. A culture chamber was defined by mounting a glass ring on the printed circuit board and sealed using Sylgard 184 (Dow Corning) silicone.

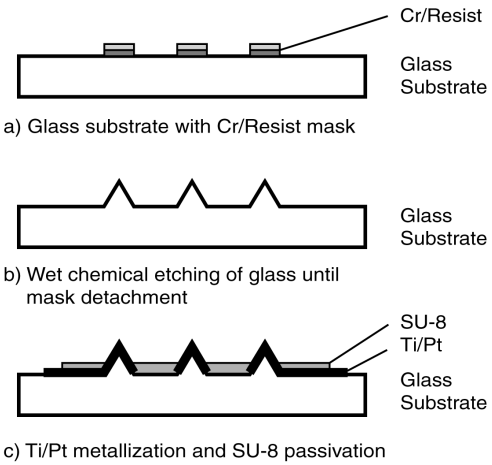

Figure 2 : Fabrication process for tip-shaped electrode arrays on glass.

III. RESULTS

A. Flexible microelectrodes with pyramidal tips

Flexible microelectrodes with a great variety of shapes (probe style and arrays) and electrode dimensions have been micromachined. The electrode sites range from 5×5 µm up to 100×100 µm resulting in pyramid heights from 3.5 µm to 70 µm. Fig. 3 shows a sharp pyramidal tip microelectrode where the pyramid is covered entirely by the platinum layer. Fig. 4 shows a flat tip electrode, which can be used to record from delicate tissue, as tissue damage is very unlikely to happen during the implantation procedure. The process technology also allows for fabricating mono- or multipolar electrode arrangements on both sides of the flexible substrate (illustrated in Fig. 1d). On one side (towards the silicon wafer) we obtain the three-dimensional, pyramidal microelectrodes or electrodes that are located at the surface (Fig. 1d, electrode type illustrated in the middle). This depends only on the mold shape of the silicon substrate. Fig. 5 shows such an electrode site directly located at the surface with a very smooth transition from the platinum to the isolating polyimide layers.

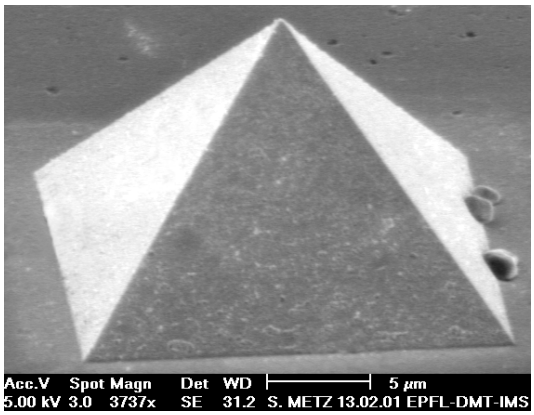

Figure 3 : Pyramidal electrode site where only the pyramid is covered with platinum for contacting tissue (corresponds to electrode type in Fig. 1d, left side).

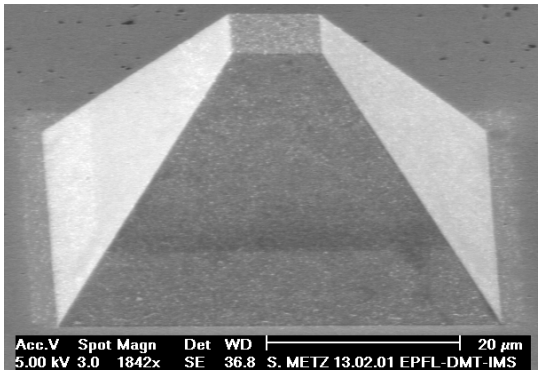

Figure 4 : Flat pyramidal electrode site for delicate tissue to reduce the damage during insertion (corresponds to electrode type in Fig. 1d, right side).

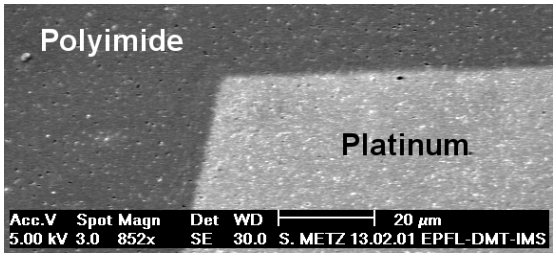

Figure 5 : Flat electrode site directly located at the surface. A smooth transition leads from the platinum covered area to the isolating polyimide (corresponds to electrode type in Fig. 1d, center).

On the other side of the flexible substrate the electrode is recessed within the polyimide layer as with the standard polyimide-platinum-polyimide sandwich technology [6].

B. Tip-shaped electrode arrays on glass

The electrode array design was adapted to a commercial signal amplification and data acquisition system (Multi Channel Systems, Germany) in order to avoid the development of external hard and software (Fig. 6). Realized electrode arrays are composed of 60 electrodes arranged in an 8×8 matrix without corners. The electrode area corresponds roughly to the lateral area of a pyramid with a side length of 40 µm and a height about 30 µm at the top of the glass tips (the global glass tip height being between 50 µm and 60 µm). The space between two electrodes (center to center) is 200 µm (Fig. 7).

First biological experiments using these 3D electrode arrays have been done on acute brain slices (Fig. 8). Current pulses are applied through one electrode (white dot) to locally stimulate the slice while the other electrodes are used for extra-cellular recording of electrical activity. Evoked neural responses recorded from rat hippocampus slices (thickness of 350 µm) showed larger signal amplitudes (in the mV range) than when using planar electrodes, which is mainly due to the larger electrode area of the three-dimensional electrodes. Moreover, the input (stimulation current on one electrode) / output (evoked signal amplitude at another electrode 200 µm apart) functions of the obtained data from planar and three-dimensional electrode arrays demonstrate

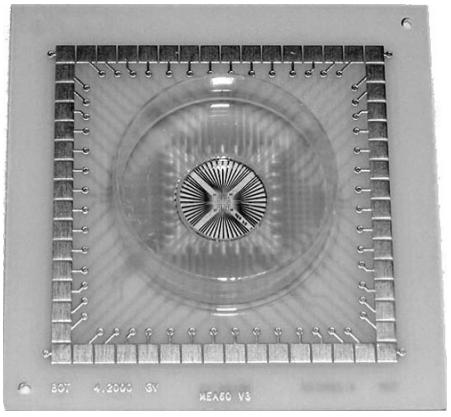

Figure 6 : Overview of a 60 electrode array after packaging. External dimensions are 5×5 cm.

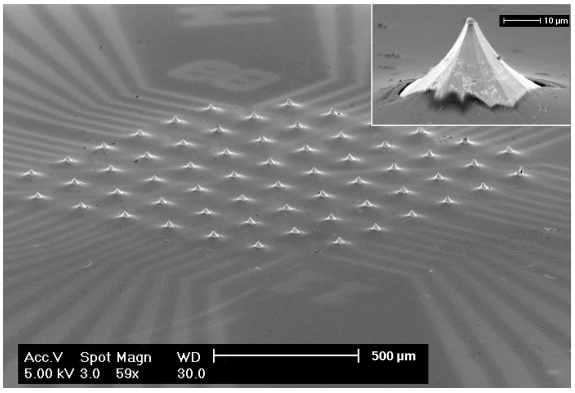

Figure 7 : Electrode array on glass substrate composed of 60 tip-shaped protruding electrodes each with a height of 60 µm. A close-up of one electrode shows the sharp tip embedded in the isolating SU-8 layer

that the 3D electrodes were closer to the active cells than in the planar configuration (Fig. 9) (data not shown).

C. Impedance Spectroscopy

The interface behavior of electrode and biological tissue was characterized 'in vitro' in a physiological saline solution by using an Impedance Analyzer (LCR meter HP 4284A). We measured the impedance of one electrode with a counter electrode of much larger surface. The measurements were carried out between 100 Hz and 1MHz with a signal of 100 mV without any bias.

As expected, an inverse relationship was found between the electrode surface area and the electrode impedance values. In addition to that, the 3D electrodes show a difference in electrode impedance due to an increase of the geometrical electrode surface when compared to flat microelectrodes. The decrease in impedance corresponds roughly to the increase in surface area. However, the phase shift yielded similar values in both measurements. Typical impedance for planar electrodes is 500 kΩ at 1 kHz for an electrode area of 40×40 µm. For both types of three-dimensional microelectrodes, we obtained impedances of 220 kΩ at 1 kHz for a tip/pyramid base area of 40×40 µm.

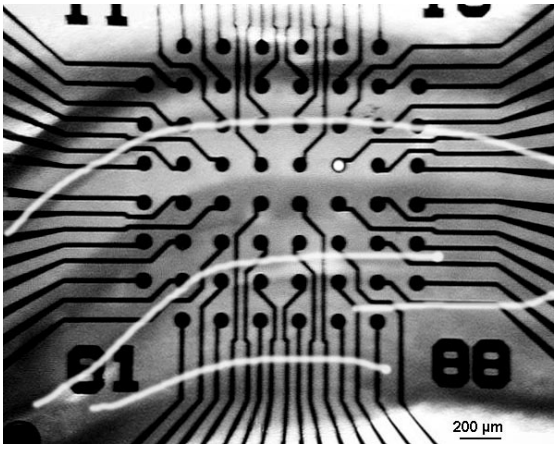

Figure 8 : Picture of a rat acute hippocampus slice placed on a 3D electrode array for electrophysiological experimentation.

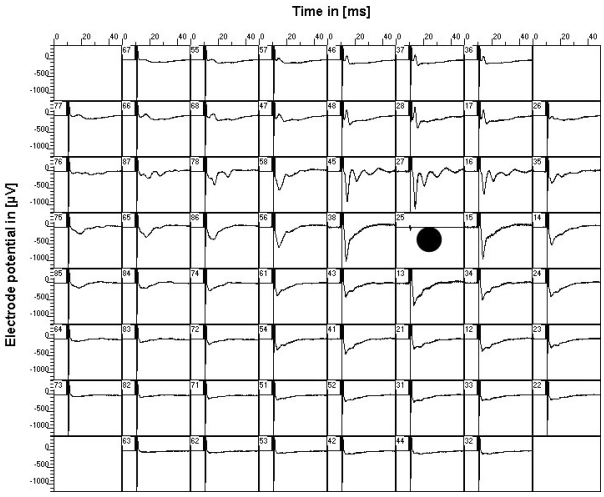

Figure 9 : Signals obtained from the acute rat hippocampus slice shown in Fig. 8. Each case of this plot represents one electrode. Schaffer collateral axons were stimulated (black dot) in the CA1 region and evoked responses of CA1 pyramidal cells were recorded.

IV. Discussion

The development of microelectrodes with integrated 3D structures was presented. The integration of three-dimensional, pyramidal structures with microelectrodes improves the contact at the electrode/tissue interface. Due to the increase in electrode surface the impedance values are decreased. However, the same selectivity with respect to electrode density and projected surface area can be reached as with flat electrodes.

For the flexible devices the fabrication processes can be carried out with photosensitive and non-photosensitive polyimide. Due to the known excellent biocompatibility, polyimide-based electrodes promise for fabrication of long-term implants for the use in prostheses. The flexible structures can be slid into tissue layers where the pyramidal tips will smoothly penetrate the tissue. For delicate tissue, the pyramids can be fabricated with flat tips. The tips can reach heights (up to 70 µm) several times higher than the thickness of the thin-film, planar substrate (typically 10 µm). Additionally, the technology provides a simple method to obtain electrode arrangements on both sides of the substrate when compared to other recently presented methods [7]. The different shapes of the electrode sides results in a wide range of selectable current density profiles when stimulating biological tissue.

The fabrication of tip-shaped electrodes in glass is simple to achieve and allows tip heights up to 100 µm depending on glass etching parameters. These types of electrodes are well suited for acute slice experimentation and improve the recording capabilities of electrode arrays.

V. Conclusion

We have developed technologies to integrate three-dimensional structures and microelectrode arrays. The versatile processes allow for a wide range of dimensions and shapes of final devices.

One fabrication procedure realizes for the first time flexible, polyimide-based microelectrodes with three-dimensional structures at the recording sites. The technology is also capable of providing electrode arrangements on the front and backside of the flexible, implantable devices, which is crucial for more complex applications when interfacing to biological tissue.

The second technology can be used with glass-based arrays to create sharp electrode tips that penetrate the dead cell layers inherently present when dealing with acute brain slices. The results suggest that 3D electrode arrays can serve as a novel tool to more precisely unravel the network properties of acute brain slice preparations.

Acknowledgment

This work was founded by the Leenaards Foundation and by a grant from the Swiss National Science Foundation (N°3152-053761.98). Clean room processing was done with the help of the staff of the EPFL Center for Microtechnology (CMI).

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